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COMPUTER-ASSISTED ANALYSIS OF SEVERAL CHEMICALS ON RABBIT SPERM CELL MOTION

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"CellSoft," a computer-assisted motion analysis system, was used to study the effect of several chemicals on the motion characteristics of sperm cells. All compounds studied decreased the percentage of motile sperm cells and reduced the average and maximum velocities of the cells in a time and concentration-dependent manner. The ED_{50} value after a 2-hr incubation was determined for all the compounds studied. The rank order of the ED_{50} values of the compounds agrees with the order of cytotoxicity determined by cell-free incorporation of radiolabeled precursors by mouse 3T3 cells. Sperm motility analysis appears suitable as a simple in vitro method for assessing the cytotoxicity potential of chemicals.

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PREFACE

The work described in this report was authorized under Project Nos. F8J210005 and 1C161102A71A, Research in CW/CB Defense. This work was started in October 1987 and completed in January 1988.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," National Institute of Health Publication No. 85-23. These investigations were also performed in accordance with the requirements of AR 70-18, Laboratory Animals, Procurement, Transportation, Use, Care, and Public Affairs.

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COMPUTER-ASSISTED ANALYSIS OF SEVERAL CHEMICALS ON RABBIT SPERM CELL MOTION

1. INTRODUCTION

A variety of chemical types are able to induce changes in the sperm cell movement characteristics of several mammalian species.[1-4] Manual quantification of the characteristics is highly subjective, slow, and imprecise. Recently, several computer-assisted systems have been developed for the rapid, objective analysis of sperm cell motion. Typically, these systems track the motion of the sperm head and provide information on motion parameters such as velocity, progression, head movement amplitude and frequency, and the motion patterns of individual sperm cells. A previous study[5] indicated that inhibition of sperm cell motility was a useful but insensitive endpoint in the in vitro assessment of chemical cytotoxicity. This report describes the use of a commercially available computer-assisted motion analyzer (CellSoft) to analyze the effects of several chemicals on rabbit sperm cell motion.

2. MATERIALS AND METHODS

New Zealand white rabbits were individually housed in standard rabbit cages. The room was maintained at 25 + 2 °C and 50 + 10% humidity with a 12/12-hr light/dark cycle. Standard approved laboratory rabbit chow and water were available _ad libitum_.

2.1 Collection of Sperm Cells.

Semen was collected with an artificial vagina.[6] The rest period between collections was at least 2 days. A log was maintained of each rabbit's semen volume and collection date.

2.2 Chemical Exposure and Incubation of Rabbit Sperm Cells.

Prostatic secretions were removed from cells by centrifugation of semen through a discontinuous Percoll gradiant.[7] Purified cells were pre-incubated in the high salt (HS) medium described by Brackett, Bousquet, and Dressel[8] and resuspended in the defined medium (DM) of Brackett and Oliphant.[9] A solution of the test compound in water, dimethyl sulfoxide, or DM was added to the cell suspension, final concentration 1.5-2.1 x 10^7/mL, and the mixture was incubated at 37 °C under an atmosphere of 5% CO_2, 8% O_2, and 87% N_2. Whenever water or dimethyl sulfoxide, or DM was used as a solvent, the same volume was added to the control cell suspension; the concentration of dimethyl sulfoxide did not exceed 1% v/v. Usually cells were exposed to three concentrations of the test compound.

2.3 Observation of Cell Motion.

At O (control only), 0.5, 1, 2, 4, and 5.5 hr, a drop of the cell suspension was placed in a 20-µm deep observation chamber, and cellular motion was recorded by videomicroscopy. The videomicroscopy system consisted of an Olympus BH2 microscope equipped with a heated stage, 10x negative-high phase contrast objective, 10x ocular, Dage NC-67M video camera, Sony VO-5800 VHS recorder, two Panasonic WV-5410 video monitors, FOR.A VTW-100 video typewriter, and a FOR.A VTG-33 time generator. Ten random fields were videotaped for each two drops of cell suspension at each time point. Each field was taped for 10-15 s. The temperature of the observation chamber was maintained at 37 °C by the heated stage and a stream of air heated to 37 °C.

2.4 Analysis of Cellular Motion.

The video images were analyzed by an IBM AT equipped with an 80287-8 math coprocessor and the "CellSoft" system proprietary hardware and software. The 10 fields of each drop were analyzed for 15 frames (0.5 s) with the software set at the threshold values recommended. These values were immobile sperm cell - 1, motile sperm cell - 2, amplitude of lateral head displacement - 7, cross beat frequency - 7, circular motion - 10, velocity range - 20-300 µm/s, and sperm head size range - 5-60 pixels.*

2.5 Chemicals.

All chemicals were the highest purity ordered from the named suppliers; ammonium oxalate (Fisher Scientific Company, Silver Spring, MD); hydroquinone, pyrogallol, and oleic acid (Chemical Service Company, Media, PA); pinacolyl alcohol, polyethylene glycol 200 (PEG), o-ethyl-o-(2-diisopropylaminoethyl) methylphosphonite [(QL) U.S. Army Chemical Research, Development and Engineering Center (CRDEC)]; and triethyl phosphite (Allied Chemical Company, New York, NY).

3. RESULTS

The motion parameter values of sperm cells differed from rabbit to rabbit. The values were even different in cells obtained from the same rabbit several days apart. Table 1 shows the range of values of six samples from three rabbits at the beginning and end of a 5.5-hr incubation period in DM at 37 °C under an atmosphere of 5% CO_2, 8% O_2, and 87% N_2. The absolute

*Pixel - a unit of monitor area defined by the magnification of microscope objective, ocular, and camera lens; a dot on the monitor screen. For the purpose of this report, 1 pixel was calibrated as 0.97 μm^2.

values of the motion parameters were different among rabbits. Nevertheless, there was some degree of consistency in the way values changed during incubation. This is illustrated in Table 2. The average and maximum velocities appear to increase after 0.5-hr incubations and then decrease after a further 2- to 3-hr incubation. The percentage of motile cells continually decreased, and the amplitude of lateral head displacement increased over the incubation period. This behavior was observed in all six sperm cell samples. In contrast, changes in cell linearity, a measure of the cell path straightness and head cross beat frequency values, were not consistent across the six rabbits. Cell linearity either decreased (n = 3) or remained essentially constant (n = 3) during incubation; whereas, head cross beat frequency values decreased (n = 3), increased (n = 1), increased then decreased (n = 1), or decreased then increased (n = 2) (Table 2).

Table 1. Range of Motion Parameter Values.* Values found in two sperm cell samples from each of three rabbits.

Time (hr)	Percent Motile	Average Velocity (μm/s)	Maximum Velocity (μm/s)	Linearity	Maximum ALH** (μm)	Average ALH (μm)	Cross B/F*** (hz)
0	44-75	50-72	99-131	8.90-9.20	1.17-1.89	0.89-1.61	13.85-16.24
5.5	13-32	55-76	83-126	7.10-8.81	1.79-2.67	1.39-2.29	12.65-16.80

*Sperm cells were incubated at 37 °C in DM under an atmosphere of 5% CO_2, 8% O_2, and 87% N_2.
**amplitude lateral head displacement (ALH)
***beat frequency (B/F)

Chemical exposure resulted in changes to the motion parameter values. All six compounds studied decreased the percentage of motile sperm cells and the average and maximum velocity in a concentration-dependent manner. The results obtained with ammonium oxalate (Figure 1) are typical changes in motion parameters. At the highest concentration (0.01M), less than 25% of the cells were motile after a 0.5-hr exposure; few cells were motile after a 2-hr exposure. Cells exposed to lower concentrations (0.00M, 0.005M) showed no decrease with respect to controls in the percentage of motile cells even after a 5.5-hr incubation (Figure 1a). All three concentrations of ammonium oxalate decreased the average velocity in a concentration-dependent manner (Figure 1b). The maximum velocity showed a

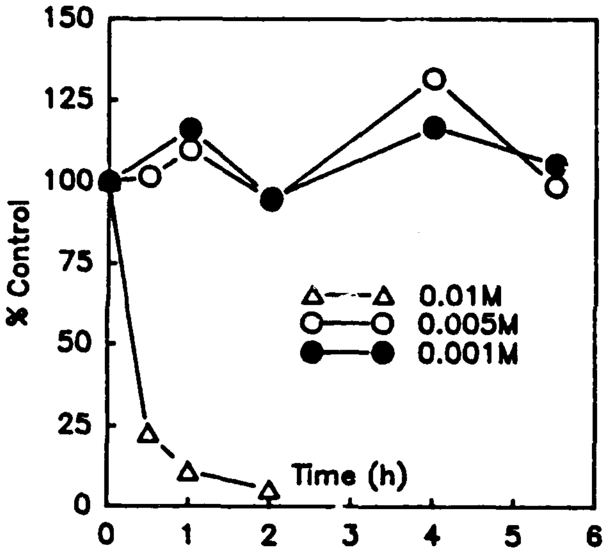
rapid drop, to about 40% of control, in the presence of 0.01M ammonium oxalate. Lower concentrations caused a slow decline to about 80% of the control at the end of a 5.5-hr incubation (Figure 1c). The decline in the number of motile sperm was rapid and was the most evident toxic effect for all six compounds. All three values dropped rapidly (Figure 2) with compounds of high toxicity (e.g., pyrogallol).

Table 2. Motion Parameters of Rabbit Sperm Cells*.

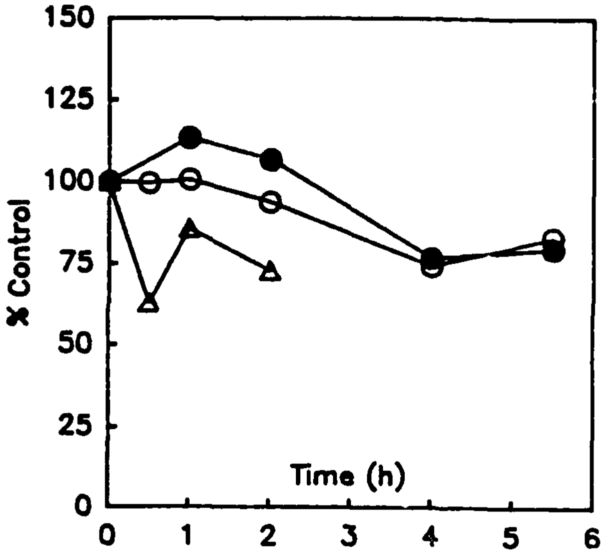
Time (hr)	Percent Motile	Average Velocity (μm/s)	Maximum Velocity (μm/s)	Linearity	Maximum ALH** (μm)	Average ALH (μm)	Cross B/F*** (hz)
0.0	57.0	71.0	123.0	9.16	1.17	1.02	16.140
0.5	53.0	58.0	135.0	9.07	1.97	1.73	14.1160
1.0	56.0	59.0	140.0	9.20	2.18	1.59	15.550
2.0	36.0	82.0	122.0	8.43	2.28	1.91	14.330
4.0	35.0	75.0	125.0	8.15	1.98	1.71	14.680
5.5	32.0	76.0	126.0	8.41	1.99	1.77	14.130

*Sperm cells were incubated at 37 °C in DM under an atmosphere of 5% CO_2, 8% O_2, and 87% N_2.
**amplitude lateral head displacement (ALH)
***beat frequency (B/F)

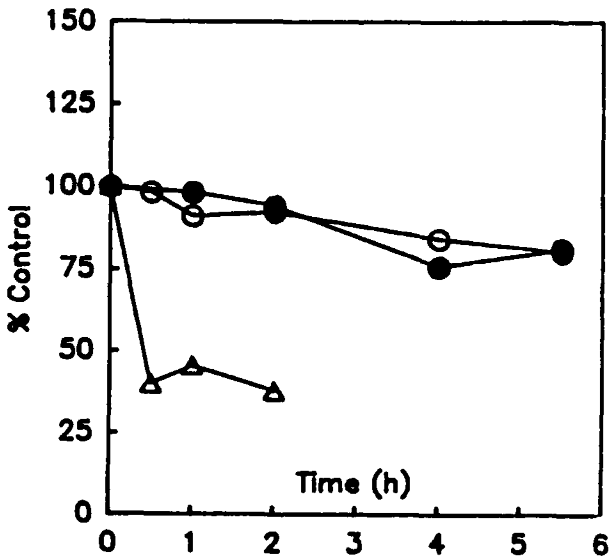
The relationship between the concentration of ammonium oxalate and percentage of motile cells, average and maximum velocity at 0.5, 1, and 2 hr is shown in Figure 3. The concentration necessary to reduce the number of motile cells and maximum velocity by 50% of controls is 8.7-9.5 x 10^{-3}M and 7.7-8.3 x 10^{-3}M, respectively. The 50% value for all six compounds is shown in Table 3. The most toxic compound of this group is pyrogallol, and the least toxic is pinacolyl alcohol. In the presence of pyrogallol, motility ceased abruptly 15 min after the compound was added.



a. Decrease in Percentage Motile

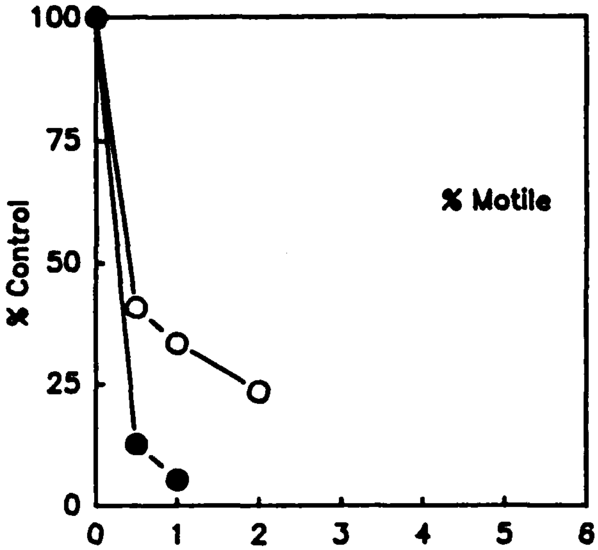


b. Decrease in Average Velocity

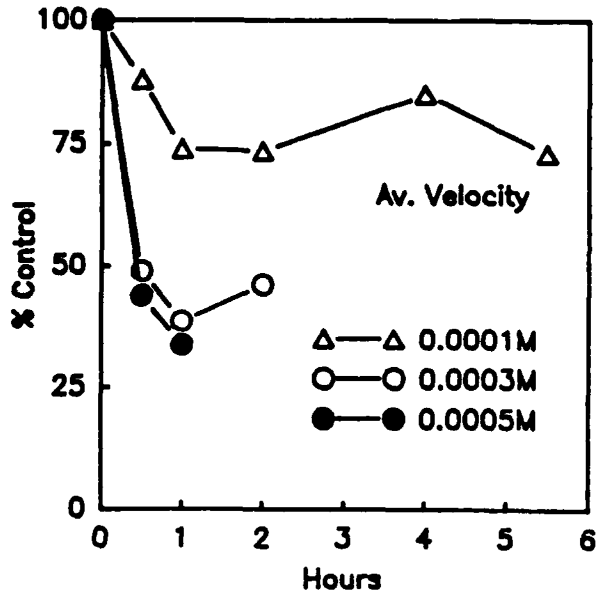


c. Decrease in Maximum Velocity

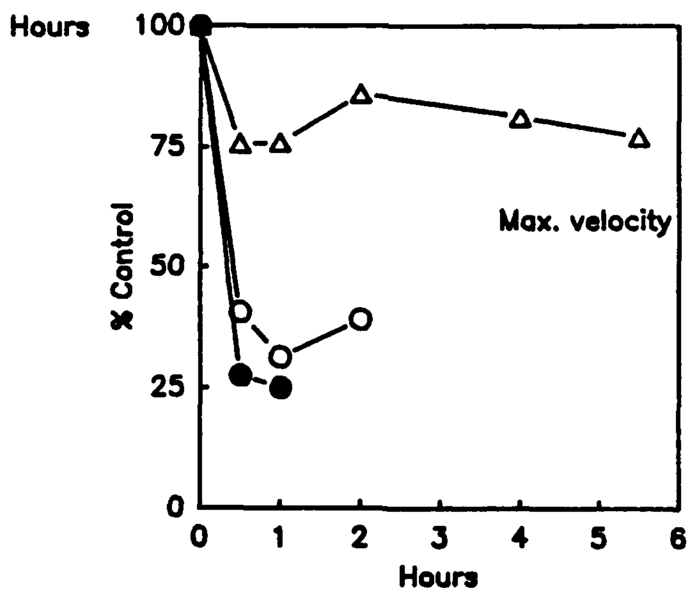
Figure 1. Effect of Ammonium Oxalate on Rabbit Sperm Cell Motion.



a. Decrease in Percentage Motile

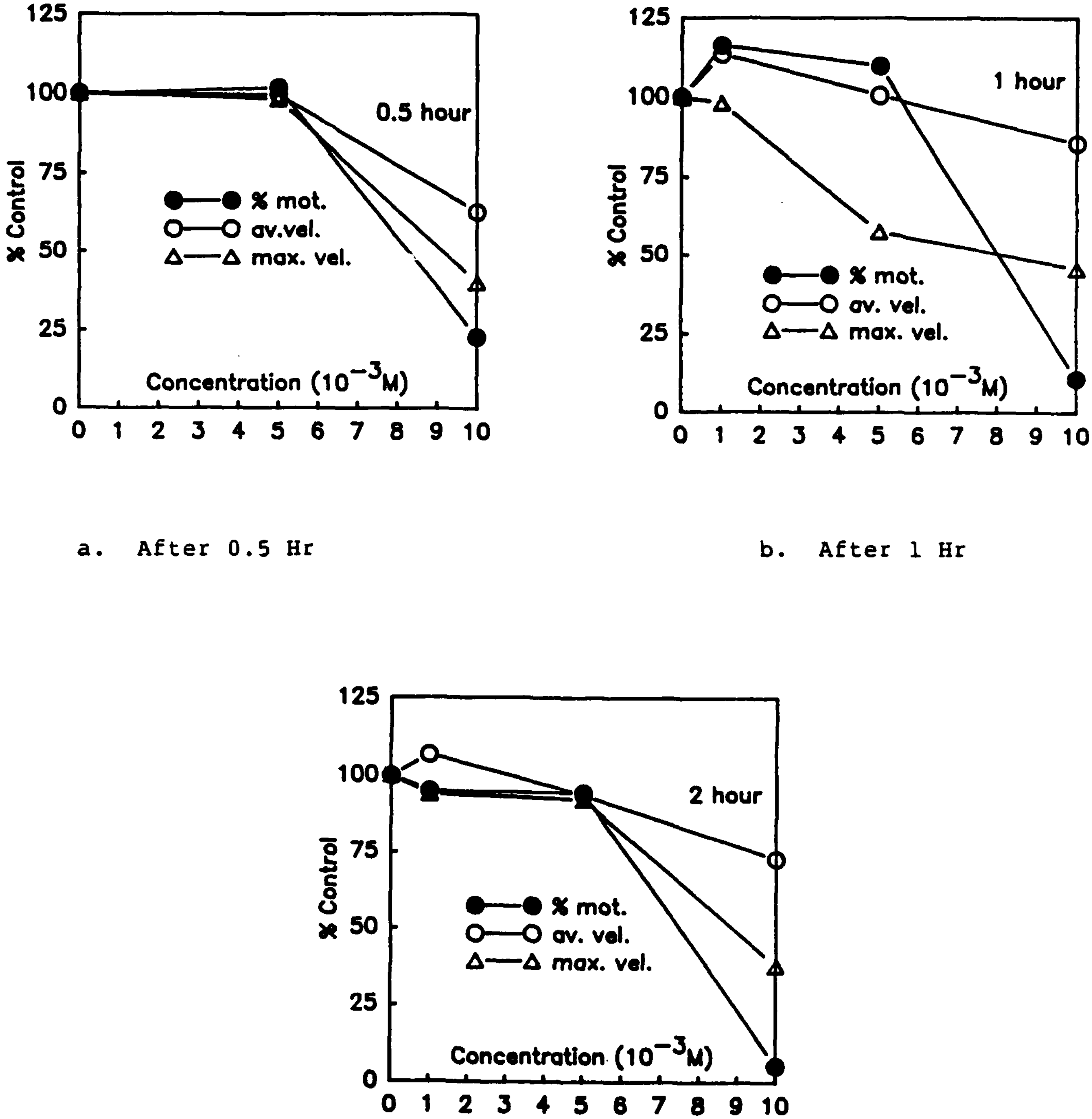


b. Decrease in Average Velocity



c. Decrease n Maximum Velocity

Figure 2. Exposure of Rabbit Sperm Cells to Pyrogallol.



a. After 0.5 Hr

b. After 1 Hr

c. After 2 Hr

Figure 3. Exposure of Rabbit Sperm Cells to Different Concentrations of Ammonium Oxalate.

Table 3. ED_{50} Values of Test Chemicals After 20-Hr Incubation.

Compound	Motility (mM)	Average Velocity (mM)	Maximum Velocity (mM)
Hydroquinone	<0.05	--	--
Pyrogallol	0.26	0.27	0.26
Oleic Acid	3.40	3.40*	3.40*
Triethyl Phosphite	4.00	5.00	4.00
Ammonium Oxalate	7.70	--	8.70
Pinacolyl Alcohol	32.00	40.0	40.00

*4-Hr Incubation

4. DISCUSSION AND CONCLUSION

Computer-assisted motion analysis is a rapid, sensitive, and objective method to quantitate motion parameters of sperm cells. The technique may also be applicable in assessing a chemical's cytotoxic potential. Table 4 shows the concentrations of the compounds required for a 50% in vitro inhibition of the growth and uridine incorporation by mouse 3T3 cells (personal communication with Dr. D.M. Stark, Laboratory Animal Research Center, Rockefeller University, New York, NY). The rank order of the ED_{50} values of the compounds (Table 3) are in excellent agreement with the concentration values required for inhibition of cell growth (Table 4). This result is not unexpected, because inhibition of sperm cell motility is essentially a reflection of cell death. There was less agreement between the rank order of ED_{50} values and uridine incorporation. Therefore, pyrogallol and triethyl phosphite were equally good in inhibiting uridine incorporation, whereas the former was 15 times more effective in inhibiting sperm motion. Because the sperm cell nucleus is inert, the disagreement is not surprising; nonetheless, the experiment should be repeated. It should be emphasized that the sperm cell motion tapes were analyzed using procedures and software settings suggested by the suppliers of the "CellSoft" system. These settings and procedures may not be appropriate for the rabbit. Therefore, the absolute ED_{50} values may not be accurate, but the compound rank of order inhibiting cell motility should be correct.

Table 4. Concentration for 50% Inhibition of Incorporation of Amino Acid and Uridine by Mouse 3T3 Cell.

Compound	Amino Acid (mM)	Uridine (mM)
Hydroquinone	0.04	0.08
Pyrogallol	0.07	0.52
Triethyl Phosphite	0.40	0.48
Ammonium Oxalate	0.50	23.00
Pinacolyl Alcohol	77.00	7.40

The present experiment shows that computer-assisted sperm cell motion analysis is able to rank compounds studied in the same order of their ability to inhibit cell growth measured by amino acid and uridine uptake in 3T3 mouse cells. Cell motion analysis has several advantages over the incorporation of radioactive precursors because the procedure is less complex, cheaper, easier to carry out, and does not require the use of radioisotopes with the attendant disposal problem. If the preliminary results presented with this report are supported by the results of more intensive studies, sperm cell motion analysis would be preferable over precursor incorporation analysis as an in vitro procedure for assessing cytotoxicity.

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